Structural aspects of the casein micelle

M. Madende & G. Osthoff
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Introduction

- **Milk**
  - complete, complex and key element for nutritional requirements of the neonate

- Serves as a medium for transport of very high concentrations of nutrients in solution

- Milk from approximately 250 of the estimated 4000 mammalian species has been analyzed <10 studied in detail

- **Milk and milk fractions**
  - characterized by a wide array of proteins, whose concentration spans across several orders of magnitude

- Different research techniques have been employed

(Dállesandro *et al.*, 2010; Martin *et al.*, 2011; Ng-Kwai-Hang, 2011)
Milk proteins

80 % Casein
($\alpha_{s1}^{-}, \alpha_{s2}^{-}, \beta^{-} \text{ and } \kappa^{-}\text{-casein})$

Peptones
(transferrin, lactoferrin, lactollin etc)

Milk proteins

16 % Whey
($\alpha$-LA, $\beta$-LG)

MFGM
(membrane proteins etc)

(D’Alessandro et al., 2010)
Caseins

- Family of phosphoproteins synthesised in the mammary gland in response to lactogenic hormones
- Caseins evolved from members of a group of SCPP
- In eutherian milks at least 3 and normally 4 gene products are found
  - $\alpha_s$1-, $\alpha_s$2-, $\beta$-, and $\kappa$-CN
- Exist as colloidal aggregates known as casein micelles
- Non-globular in structure – No cysteine residues
- Refractory to crystallization
- Amphipathic in nature

(Horne, 2011; Mather, 2011; Holt, 2014)
**$\alpha_{s1}$ - Casein**

- Major casein fraction of bovine milk
- Precipitate in Ca$^{2+}$
- Highly conserved signal peptide
- Highly phosphorylated → 2 forms
- Contain multiple phosphorylation sites (primary and secondary)
- Several variants exist (A, B & C forms)

(Ginger & Grigor, 1999; Horne, 2011; Mather, 2011; http://www.ebi.ac.uk/clustaO)
### $\alpha_s^2$-Casein

- Highly and variably phosphorylated
  - 4 differentially phosphorylated isoforms
- Genes closely related to $\beta$-casein
- Least hydrophobic
- Highly conserved signal peptide
- Absent in human milk (pseudogene present)

<table>
<thead>
<tr>
<th>Species</th>
<th>Alpha S2 Casein Sequence</th>
</tr>
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<tbody>
<tr>
<td>Pig</td>
<td>MKIFFITCLLAVAAKHEMEHVSSSSESINISQEKYKQEKNVINHPSKEDICATSCETVEV</td>
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<tr>
<td>Cow</td>
<td>MKIFFITCLLAVAAKNTMEHVSSSESI-ISQETYKQEKRMAINPSKENDEDCTFCEEV</td>
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<tr>
<td>Goat</td>
<td>MKIFFITCLLAVAAKHMEHVSSSEEPINIFQEIEYKQEKMAIHPRKEKLCTTSCEEV</td>
</tr>
</tbody>
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(Ginger & Grigor, 1999; Horne, 2011; Mather, 2011; http://www.ebi.ac.uk/clustaO)
**β-Casein**

- Most hydrophobic casein
- Characteristic high proline
- Possess a very acidic N-terminal sequence (Glu)
- Hydrolysis by plasmin yields γ-casein
- Highly conserved 15 aa signal peptide
- One phosphorylated form and 3 variants in Bovine

(Ginger & Grigor, 1999; Horne, 2011; Mather, 2011; http://www.ebi.ac.uk/clustalO)
κ-Casein

- Most studied casein (involved in milk clotting)
- Insensitive to Ca\textsuperscript{2+} precipitation
- Only casein that is glycosylated (Thr and Ser)
- Conserved 21 aa signal peptide
- N-terminal (+ve), C-terminal (-ve)
- Target for chymosin (Phe-Met/Ile/Leu)
- 2 variants of bovine κ-CN (A & B)

(Ginger & Grigor, 1999; Horne, 2011; Mather, 2011; http://www.ebi.ac.uk/clustaO)
CASEIN MICELLES & MODELS
Casein micelle

- Exist in milk as aggregates with colloidal calcium phosphate → micelles
- A typical bovine micelle consists of $10^4$ casein molecules
- Caseins are held together by hydrophobic and electrostatic interactions
- Micelles are stabilized by a layer of κ-casein

(Ginger & Grigor, 1999; Farrell et al., 2006; Holt, 2013)
Casein micelle models

- The casein micelle occupies a unique position among biological systems so much so that various different models have been proposed for its structure.

- This situation has probably developed due to:
  - Relatively large size of the casein micelles
  - Inability to crystallize
  - Easily perturbed
  - Experimental data covering a limited range of micellar properties

- Most of the proposed models fall into three general categories:
  - Coat-core
  - Sub-micelles
  - Internal structure

(Phadungath, 2005; Holt, 2013)
Coat-core model

(Waugh & Nobel, 1965; Wong, 1988)
Sub-micelle model

A: a submicelle; B: protruding chain; C: Calcium phosphate; D: κ-casein; E: phosphate groups

(Walstra, 1984; Farrell, 2011; Horne, 2011)
Internal structure model

(Horne, 1998)
BIOLOGICAL FUNCTION
1. Safe secretion of high concentrations of calcium and phosphate so that the mammary gland does not become calcified

2. Safe secretion of high concentrations of potentially fibrillogenic casein proteins through the mammary gland

3. Retention of micelles in the stomach of the neonate so that nutrients can be digested and absorbed

(Farrell et al., 2006; Holt, 2013)
Biological function

Endoplasmic reticulum | Golgi apparatus | Secretory vesicle | Alveoli, ducts | Cisterns | Neonate stomach

Synthesis → Association

Casein micelle formation → ACP sequestration

Amyloid fibrils | Calcified deposits | Weak or no gelation

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(Farrell et al., 2006; Holt, 2013)
AFRICAN ELEPHANT CASEINS
Caseins of African elephant milk

pI 5-8

<table>
<thead>
<tr>
<th>kDa</th>
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<tr>
<td>116</td>
</tr>
<tr>
<td>66.2</td>
</tr>
<tr>
<td>45</td>
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<td>25</td>
</tr>
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<td>14.4</td>
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β-casein  β-casein  β-casein  κ-casein
Conclusions

No alpha caseins → African elephant milk the only characterized milk that lacks $\alpha_s1$- and $\alpha_s2$-caseins.

Ratio of $\beta$- to $\kappa$-casein approximately $8.5:1$.
What is the impact of the absence α-caseins on African elephant milk casein micelles?
Approach

- Extreme-resolution Analytical Field Emission SEM

- Observe the finest structural morphology of nanomaterials at 1,000,000X magnification with sub-1nm resolution

- Image thin, electron transparent samples with sub 0.8 nm resolution using an optional retractable STEM detector
RESULTS
Casein micelle

Bovine casein micelle

African elephant casein micelle

(Dalgleish, 2004)
Casein micelle

African elephant casein micelles

A

B
Conclusions

- The exact structure of the casein micelle is still not clear and therefore several models exist which attempt to describe its structure, non-bovine caseins have not been studied extensively.

- Elephant caseins are unique, they display several distinct characteristics and proportions compared to caseins of bovine and other mammals.

- The study of unique caseins may shed more light on the casein micelle structure in general and perhaps provide means to development of casein based functional foods.

- The absence of alpha caseins in African elephant milk seem to affect the size of casein micelles which appear to be much bigger in size.
Thank You
Dankie
Tinotenda